Evaluation of Ambient Ozone Injury On the Foliage of Vegetation in the Cape Romain National Wildlife Refuge South Carolina 2003 Observations

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INTRODUCTION

The Cape Romain National Wildlife Refuge (NWR) is one of more than 500 refuges in the National Wildlife Refuge System (NWRS) administered by the U.S. Fish and Wildlife Service (FWS). The NWRS is a network of lands and waters managed specifically for the protection of wildlife and wildlife habitat and represents the most comprehensive wildlife management program in the world. Units of the system stretch across the United States from northern Alaska to the Florida Keys and include small islands in the Caribbean and South Pacific. The character of the refuges is as diverse as the nation itself.

Cape Romain NWR is located in Charleston County, South Carolina,, and contains 64,229 acres that are managed to provide quality habitat for a diversity of wildlife species. Congress has conferred wilderness status on 28,000 acres of the Cape Romain NWR.

Objectives

1). To identify ozone–sensitive plant species in the Cape Romain NWR

2). To evaluate the incidence and severity of ozone injury on vegetation in the NWR

Justification

Cape Romain Wilderness has been designated a Class I air quality area, receiving further protection under the Clean Air Act. Congress gave FWS and the other Federal land managers for Class I areas an "…affirmative responsibility to protect all those air quality related values (including visibility) of such lands..." Air quality related values include vegetation, wildlife, water, soils, visibility, and cultural resources. Despite this special protection, many of the resources in these wilderness areas are being impacted, or have the potential to be impacted, by air pollutants. Because many air pollutants can be carried long distances, even remote areas such as wilderness areas can be affected by air pollution. To better understand how air pollution affects resources at the Cape Romain NWR, D.D. Davis conducted previous surveys in 1996-1998 and 2002 to evaluate ozone injury to vegetation within the refuge. The investigator conducted this ozone-injury survey in 2003.

Diagnosis of Air Pollution Injury on Plants

Although many gaseous air pollutants are emitted into the atmosphere, only certain ones are phytotoxic and induce characteristic leaf symptoms that are useful during field surveys. The most important of these gaseous, phytotoxic air pollutants are ozone, sulfur dioxide, and fluorides. These pollutants, along with the normal constituents of the air, are taken into the plant leaf through the stomata. Once inside the leaf, the pollutant or its breakdown products react with cellular components causing tissue injury or death.

The resulting macroscopic symptoms, which are visible on the leaf surface, are classified as chronic or acute depending upon the severity of injury. Chronic symptoms imply tissue injury, whereas acute injury signifies tissue death. Chronic symptoms on foliage usually result a plant's exposure to low levels of pollution for an extended time, or occur when a plant is somewhat resistant to a pollutant. Visible ozone injury is usually considered to be chronic injury. Acute injury may be observed following a short-term, high concentration of pollution, or occurs when a plant is in a very sensitive condition. Sulfur dioxide injury as observed in the field is often acute.

Macroscopic leaf injury caused by air pollutants often represents an intermediate step between initial physiological events and decreases in plant productivity. Decreases in plant productivity (Pye 1988) may result in ecological changes, such as reduced diversity (Rosenberg et al. 1979). Visible leaf symptoms induced by phytotoxic pollutants serve as important diagnostic tools that allow observers to identify specific air pollutants as causal agents of vegetation damage (Davis 1984; Skelly et al. 1987, Skelly 2000). This knowledge can be used in the air pollution emissions permitting process for siting new industries (i.e. Prevention of Significant Deterioration Program), assessment of the secondary air quality standards, assessing the presence of air pollution injury in Class I areas, and in litigation involving air pollution injury.

Although ozone was the air pollutant of concern in this survey, it should be recognized that phytotoxic levels of air primary pollutants such as sulfur dioxide and fluorides might occur near industrial sources. Likewise, trace elements including metals may be found in excessive levels in vegetation growing in areas downwind from industrial or urban sources (Davis et al. 1984, Davis et al. 2001). Toxic elements such as arsenic, mercury (Davis 2002), and lead may be especially important in areas being managed for wildlife. Although such compounds are of more interest in mammalian and avian toxicity as compared to phytotoxicity, vegetation may sorb such contaminants and become part of the contaminated food chain. However, the presence of excessive elements such as metals, as well as organic biohazards such as dioxins and furans, is determined with laboratory analysis of foliage, not with surveys dealing with macroscopic foliar injury.

Ozone

Ozone is probably the most important and widespread phytotoxic air pollutant in the United States, and is the air pollutant most likely to have an easily recognizable impact on vegetation within a NWR. Background levels of ozone exist naturally in the lower atmosphere, possibly originating from vertical downdrafts of ozone from the stratosphere, lightning, or chemical reactions of naturally occurring precursors. However, in many areas, precursors leading to phytotoxic levels of ozone originate from upwind urban areas. In those areas, hydrocarbons and oxides of nitrogen are emitted into the atmosphere from various industrial sources and automobiles. These compounds undergo photochemical reactions in the presence of sunlight forming photochemical smog, of which ozone is a major component. Ozone, or its precursors may travel downwind for hundreds of miles during long-range transport, as influenced by wind direction and movement of weather fronts. Thus, ozone impinging on refuges may originate in areas many miles upwind from the refuge. In fact, concentrations of ozone are often greater in rural areas downwind from urban areas, as compared to within an upwind urban area, due to the presence of reactive pollutants in the urban air that scavenge the ozone.

There are certain bioindicator plants in the East that are very sensitive to ozone and exhibit characteristic symptoms when exposed to ozone (Anderson et al. 1989, Davis and Coppolino 1976, Davis and Skelly 1992, Davis et al. 1981, Davis and Wilhour 1976, and Jensen and Dochinger 1989). The principal investigator in this survey routinely uses the following broad-leaved bioindicator species for evaluating ozone injury: black cherry (Prunus serotina), common elder (Sambucus canadensis), common milkweed (Asclepias syriaca), grape (Vitis spp), white ash (Fraxinus americana), and yellow-poplar (Liriodendron tulipifera). The investigator also uses, but less commonly, Virginia creeper (Parthenocissus quinquefolia) and Viburnum spp.

Ozone-induced symptoms on broadleaved bioindicators usually appear as small 1 - 2 mm diameter "stipples" of pigmented, black or reddish-purple tissue, restricted by the veinlets, on the adaxial surface of mature leaves (see Skelly 2000, Skelly et al. 1987). Immature leaves seldom exhibit symptoms, whereas premature defoliation of mature leaves may occur on sensitive species. To the casual observer, these symptoms are similar to those induced by other stresses (e.g., nutrient deficiency, fall coloration, heat stress, as well as certain insects, and diseases). However, the pigmented, adaxial stipple on plants of known ozone-sensitivity (i.e., black cherry or grape) is a reliable diagnostic symptom that can be used to evaluate ozone injury.

On eastern conifers, the most reliable symptom (current-year needles only) induced by ozone is a chlorotic mottle, which consists of small patches of chlorotic tissue interspersed within the green, healthy needle tissue. The mottle usually has a "soft edge" (as opposed to a sharply defined edge) to the individual mottled areas. An extremely sensitive plant may exhibit needle tip browning. However, this latter symptom can be caused by many stresses and therefore is not a reliable diagnostic symptom. Conifer needles older than current-growing season needles are not useful as monitors, since over-wintering and multi-year insect injuries may produce symptoms similar to that caused by ozone. Ozone injury to monocots, such as grasses (i.e., Spartina sp.), is also very difficult to diagnose in the field, as there are many causal agents that can result in tipburn and chlorotic mottle on grasses.

Description of the Refuge
(Adapted from FWS Information)

Cape Romain National Wildlife Refuge was established in 1932 as a migratory bird refuge. Bull Island was purchased and added to the refuge in 1936. The refuge is located in northeastern Charleston County, South Carolina, 20 miles north of Charleston, and lies east of U.S. Route 17 and south of the Santee River. The refuge encompasses a 20-mile segment of the Atlantic coast, including an expanse of barrier islands, salt marshes, intricate coastal waterways, long sandy beaches, fresh and brackish water impoundments, and maritime forest. The refuge headquarters is located on seven acres of permitted lands within the Francis Marion National Forest. The headquarters and maintenance shop at Garris Landing (formerly Moores Landing) are the only mainland sites. The remainder of the refuge is accessible only by boat.

The land area consists of 34,229 acres, 28,000 of which are preserved within the National Wilderness Preservation System. The refuge is also part of the Carolinian-South Atlantic Biosphere Reserve. The refuge's original objectives were to preserve in public ownership habitat for waterfowl, shorebirds, and resident species. In recent years, objectives have also included the management of endangered species, protection of the Class I Wilderness Area, preservation of the Bull Island forest with its diverse plant community, and to provide environmental education and recreation for the public. The refuge currently receives approximately 160,000 visitors annually.

The salt marshes of Cape Romain are interlaced by waterways that create a score of islands, some so low they are inundated at high tide. Others, like Cape and Bull Island are higher and not usually covered by tides. The refuge consists of acres of beach and sand dunes, salt marsh, maritime forests, tidal creeks, fresh and brackish water impoundments, and 30,000 acres of open water. Cape Island's trees are mainly pines and myrtles, while the Bull Island forest consists of live oaks (Quercus virginiana), magnolias (Magnolia spp.), pines (Pinus spp.), and palmettos (Sabal spp.).

Bull Island, an ancient barrier reef, is the most visited part of the refuge. Low and rolling, about 6 miles long and 2 miles wide, it lies nearly 3 miles off the mainland and is reached by boat from Garris Landing. The broad, open beach is shell-strewn and seems to stretch endlessly north and south. Over the centuries, the ocean has washed away a lighthouse, a cape, and many acres of forest. Inland are woods and large ponds. Wintering waterfowl heavily use these ponds; in spring, wood duck families nest in surrounding trees. Concentrations of waterfowl, shore birds, wading birds, and raptors abound, with over 337 bird species found on the refuge. The refuge contains the largest nesting rookery for brown pelicans, terns, and gulls on South Carolina Coast. In addition, Cape Romain NWR has the largest nesting population of loggerhead sea turtles outside the State of Florida, and currently plays an integral role in recovery of the endangered red wolf.

The refuge is rich in the history of South Carolina. Seewee Native Americans inhabited the area before the arrival of European settlers. The tidal creeks and bays provided the natives with ample supplies of fish, oysters, and clams. Several Native American's shell middens are located on the refuge. English settlers in South Carolina made their first landing in the New World on Bull Island to resupply their stocks of wood, water, and food before proceeding further south. They eventually established the first permanent European settlement in South Carolina at the present location of the City of Charleston.

Bull Bay and the creeks behind Bull Island were reputed hideouts for pirates plundering ships along the coast. The remains of the "Old Fort" on Bull Island are believed to have been a Martello tower built in the early 1700's. There are documented stories of retreating British warships restocking supplies on Bull Island during the Revolutionary War, Confederate blockade runners using refuge tidal creeks, and the Union troops' destruction of the Martello tower used as a Confederate powder magazine. Two lighthouses, built in 1827 and 1857, still stand on Lighthouse Island. Although neither is operational, they are still used as daytime landmarks for ships and fishermen.

Results of Surveys by Others

Previous surveys were conducted during the summers of 1986 and 1988 to document the presence or absence of foliar injury possibly caused by ambient ozone on vegetation within the Cape Romain NWR. Anderson et al. (1986) surveyed five plots in 1986 on the eastern half of Bull Island. He reported that 100% of 87 Chinese tallowtrees exhibited symptoms resembling those caused by ozone. However, they stated, “We do not have any fumigation data on this species and cannot be sure if the observed symptoms were ozone caused.” They also suggested that future surveys be conducted in late August rather than in September, to avoid having to contend with confounding fall coloration on some species.

Based on a 1988 field survey on all three islands (Bull, Cape, and Lighthouse Islands) in the refuge, Zedaker et al. (1990) reported that nine of eleven bioindicator species examined exhibited “ozone injury” symptoms. Unfortunately, these authors considered nearly any form of foliar discoloration as being caused by ozone, making their report useless in terms of evaluating ozone injury.

During annual refuge surveys in 1996-1998 and 2002, D.D. Davis found typical ozone injury on wild grape and Chinese tallowtree within the Cape Romain NWR. Winged sumac also exhibited ozone symptoms, but severe foliar reddening complicated evaluation of sumac foliage.

METHODS

General Survey Locations

It had been predetermined that survey sites had to occur in open-areas (such as those occurring along roads or trails, or in fields), where ozone-sensitive plant species were found in sunlight and exposed to unrestricted air movement (Anderson et al. 1989; USDA Forest Service, 1990). Immediately prior to the first survey in 1996, maps of the refuge were viewed with refuge personnel, in order to select tentative survey areas. Based on these initial discussions, preliminary survey areas throughout the refuge were selected in 1996. Each area was visited, and its suitability as a survey site was determined in the field during the 1996 visit. Survey sites, tentatively located in the upland area near Garris Landing (formerly Moores Landing) and on the three refuge islands (Bull Island, Lighthouse Island and Cape Island), were visited in 1996. However, the latter two islands contained few bioindicator species and were not surveyed after the initial year.

In 2003, two general locations were surveyed (Figures 1, 2). The first survey location was located near the boat launch at Garris Landing ("1" on Figure 1). Elevations in this area ranged from sea level to 20 feet. The other survey location was Bull Island ("2" on Figure 1, Figure 2), where the elevation ranged from sea level to 10 feet, and which could be reached only by boat. Bull Island has an unimproved road system that formed the basis of the survey route in 2003. Both these areas had been surveyed in 1996-1998 and 2002.

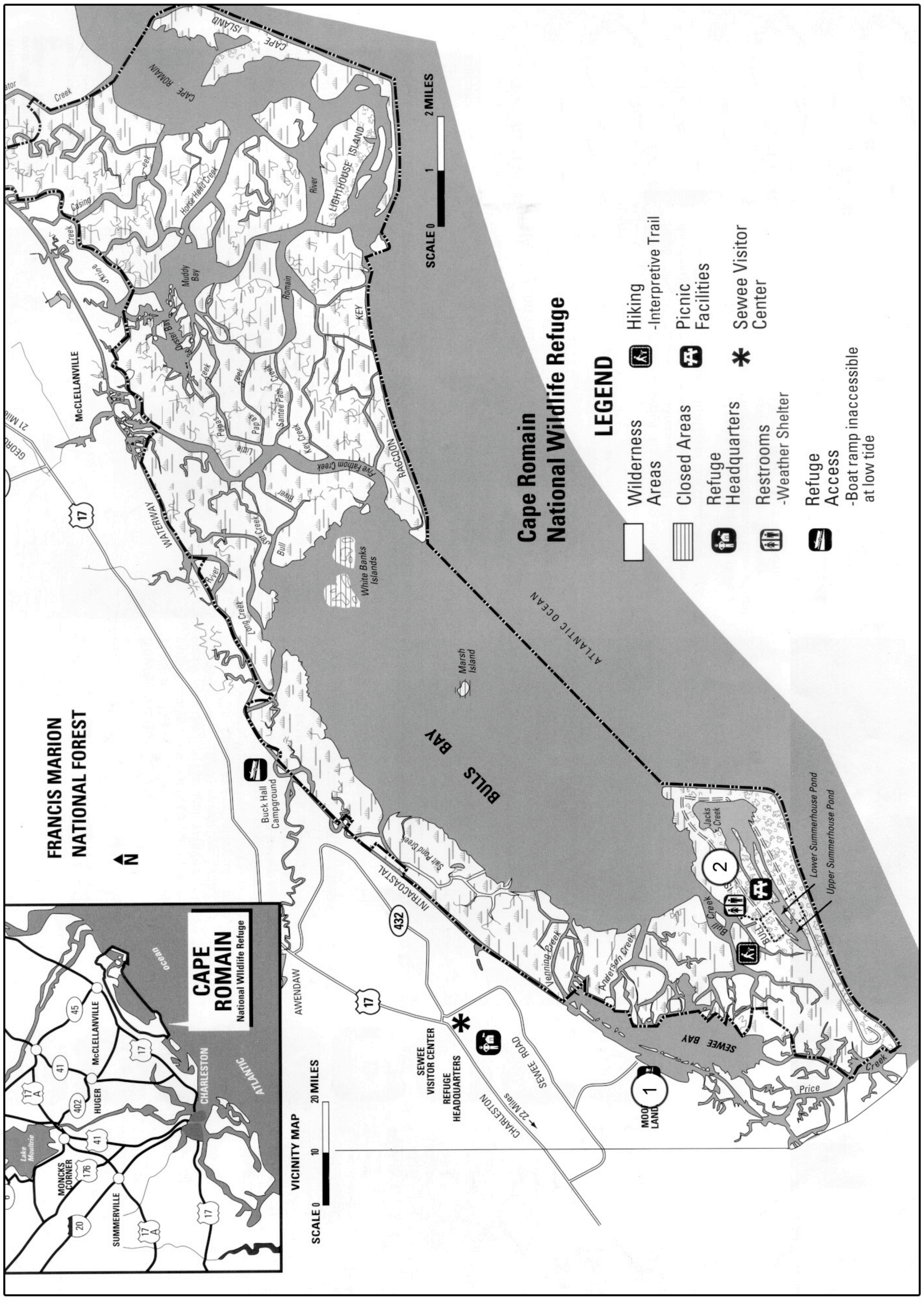

Figure 1. Location of the Garris Landing ("1") and Bull Island ("2") within the Cape Romain National Wildlife Refuge (See text for exact survey site locations).

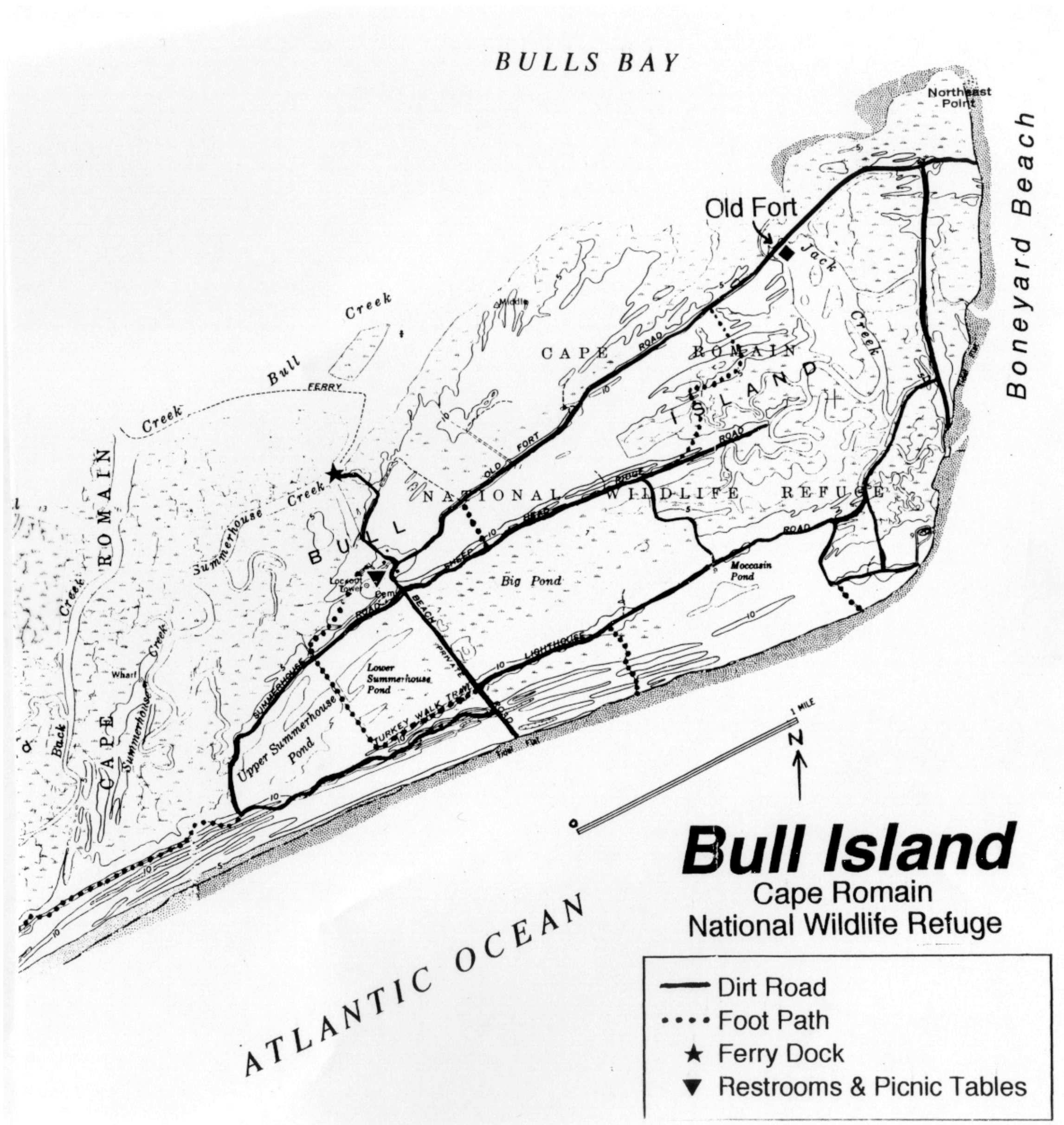

Figure 2. Location of the unimproved road system that served as survey routes on Bull Island within the Cape Romain National Wildlife Refuge.

Selection of Bioindicator Species

A botanical survey of the flora of Bull Island was conducted between 1973 and 1975, and 268 species of native and introduced plants were identified (Stalter, 1984). In addition, Helm et al. (1991) conducted a survey in 1989 to characterize the maritime forest on Bull Island. (For a combined list of plant species, see Appendix). Hurricane Hugo changed the vegetative complexion of the area when a 20-foot storm surge accompanying the hurricane swept over some of the islands in September 1989. The hurricane killed much of the overstory on Bull Island, changing it from a full canopied, forested island to a brushy island with a limited canopy. The hurricane was responsible for killing most, if not all, of the loblolly pines present at the time on Bull Island. (Pine seedlings later emerged, apparently from the seed bank in the soil). Thus, there may be differences in vegetation surveys, especially as related to species abundance, when comparing the results of "pre-Hugo" vs "post-Hugo" surveys.

Possible bioindicators, known to be sensitive to ozone, were selected from the lists of the botanical surveys. Potential bioindicators chosen prior to the 1996 survey included black cherry, Chinese tallowtree, loblolly pine, muscadine grape (Vitis rotundifolia), poison-ivy, red bay, Rubus spp., Smilax spp., salt marsh cordgrass, sweetgum, trumpet vine (Campsis radicans), Virginia creeper, wild grape (Vitis sp.), and winged sumac (Rhus copallina).

Many of these species grow in scattered localities through the NWR, and may not be present at desired survey areas; they may only be found with the help of local botanists. Also, most plant species growing in the wetter parts of the refuge have not been studied with regard to ozone-induced macroscopic symptoms. That is, the ozone-sensitivity of wetland species, as determined by controlled exposures of ozone, is generally unknown (nor is the ozone-sensitivity of the spring ephemerals, which are no longer present by the time of late summer surveys).

Based on observations made during the 1996-1998 field surveys, bioindicators to be used in 2003 included mainly black cherry, Chinese tallowtree, Rubus spp, sweetgum, wild grape, and winged sumac.

Air Quality

Ozone monitoring data are useful to help understand and interpret the results of visual injury surveys. In general, as long as soil moisture is adequate and temperatures are moderate, ozone-induced stipple is likely to be greatest in years with greatest ozone concentrations. However, more consistent and long-term monitoring datasets are needed to characterize or model the relationship between stipple and factors such as ambient ozone level, time of year, plant species, and environmental conditions (e.g., soil moisture) in our national parks and refuges.

Ambient ozone is monitored within the Cape Romain NWR at EPA AIRS site 45-019-0046. In this report, ambient ozone levels in this report are expressed as “cumsum60”, the cumulative sum of all hourly ozone concentrations equaling or exceeding 60 ppb, expressed as ppb.hrs. In other studies, we have found that this ozone statistic correlates with plant stipple from ozone.

During the first 3 years of surveys by the investigator, ozone levels were greatest in 1997, least in 1996, and intermediate in 1998 (Figure 3). The cumsum60 level of ozone during these 3 years peaked at 30,000 ppb.hrs, which occurred by October 1997; however, this peak occurred after the field survey had been conducted. Ozone levels by the end of August are more reflective of the ozone stress that vegetation has encountered at survey time. By late August, the approximate cumsum60 levels (ppb.hrs) reached the following moderate levels: 1996 (9,000), 1997 (27,000), 1998 (22,000), 2002 (17,000), and 2003 (18,000).

Ambient ozone monitoring has revealed that the Cape Romain NWR experiences ozone levels similar to other rural refuges such as the Moosehorn NWR in Maine, but much lower than ozone levels at the Mingo NWR in Missouri, Edwin B. Forsythe NWR in New Jersey, and Wichita Mountains NWR in Oklahoma. For example, the ozone levels at the Edwin B. Forsythe NWR reached about 80,000 ppb.hrs in 1991 (a very high ozone year), 60,000 ppb.hrs in both 1997 and 1998, and are often greater than 40,000 ppb.hrs by summer’s end. During 1999, ozone levels at the Mingo NWR likewise reached 80,000 ppb.hrs by late fall. In Oklahoma, ozone levels are routinely greater than 40,000 ppb.hrs by fall; in addition, the more southerly refuges are likely to experience measurable ambient ozone much earlier in the spring, and for a longer period of time, than at the more northerly refuges. Correspondingly, vegetation at the southern refuges may be exposed to greater accumulation of ozone dose during the summer.

Previous ozone levels monitored at the Cape Romain NWR in 1996-1998 and 2002 were relatively low, as compared to the extreme ozone levels at refuges such as Brigantine, but are still high enough to be phytotoxic to sensitive species of vegetation, as borne out by previous years' survey results. The ambient levels of ozone at Cape Romain NWR are great enough to cause injury on very sensitive plant species such as wild grape and Chinese tallowtree during most years. It is likely that the ozone levels in this refuge are great enough to cause plant injury in the Class I Wilderness area in most years.

Time of Surveys

The Cape Romain NWR had been previously surveyed for ozone injury by the investigator during August 21-24 (1996), August 13-18 (1997), August 18-20 (1998), and September 9-11 (2002). In 2003 the refuge was surveyed during September 3-5.

Specific Survey Sites

Two general areas were to be surveyed in 2003: the upland site of Garris Landing, and Bull Island within the Class I area (Figure 1). Within these areas, specific survey sites had been selected during previous surveys. The 2003 survey area at Garris Landing consisted of an upland maritime forest with a dense canopy. Because of the dense canopy, specific survey sites had to be restricted to edge of the openings surrounding the access roads and boat landing. Presence or absence of sensitive species dictated specific survey sites.

The forest of Bull Island had a fairly open canopy, and bioindicator plants were often exposed to full sunlight and unrestricted air movement. Therefore, the presence or absence of bioindicator species determined the location of specific survey sites on Bull Island. In 2003 the entire road network (Figure 2) of the island was first traversed, as in past years. Then, several additional trips around the island were made, during which time surveys were conducted at specific sites where suitable numbers of bioindicator plants were growing.

At each survey site, plant species were examined to determine if they exhibited typical ozone injury symptoms (i.e., adaxial stipple). Plants of unknown taxonomy were also examined in a cursory manner to determine if they exhibited adaxial stipple similar to that caused by ozone.

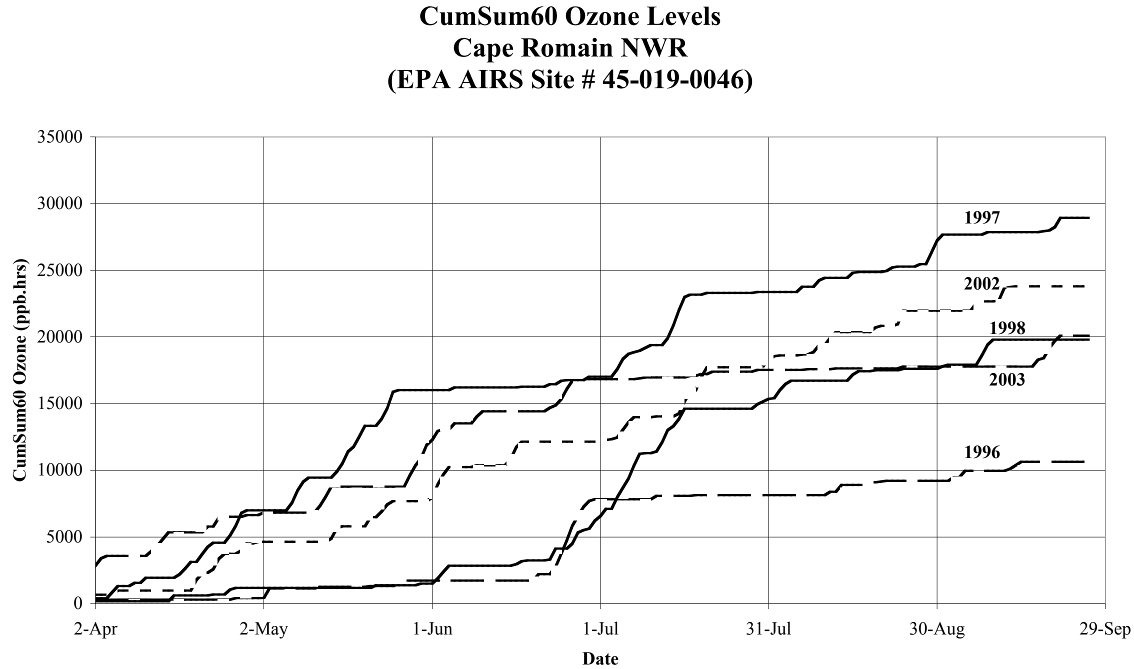

Figure 3. Cumulative sum of all hourly ozone concentrations equaling or exceeding 60 ppb (cumsum60, ppb.hrs) monitored within the Cape Romain refuge at EPA AIRS Site # 45-019-0046 during years of survey (1996-1998 and 2002-2003).

Severity Rating

Each broadleaved plant evaluated for ambient ozone injury had to have foliage within reach; that is, trees were not climbed nor were pole-pruners used. The main dataset taken was the number of plants (presented as a percentage) within a species that exhibited stipple.

In addition, the percentage of leaf tissue injured by ozone was estimated on one or two selected bioindicators. The ForestHealth Expert System had been used to train the investigator in estimating the amount of stipple on a leaf. For broadleaved tree species, the percentage of ozone injury was estimated on the oldest leaf on each of four branches, and the average value recorded. Then, the next oldest leaf was evaluated, and so on, until the five oldest leaves had been rated. For each herbaceous plant, each of the five (if present) oldest (basal) leaves of the plant was examined and the average percent stipple recorded. Each of the oldest five leaves on the current woody growth (canes) of vines was rated and the average percent stipple recorded. On all species, only adaxial leaf surfaces were evaluated. Symptom severity on the adaxial surface of each leaf evaluated was estimated by assigning severity classes, based on the percentage of surface injured, of 0, 5, 10, 20, 40, 60, 80, 90, 95 and 100 %.

Photographs (slides) were taken and originals sent to the FWS Air Quality Branch in Denver.

RESULTS

Final Selection of Bioindicator Species

As in past years, the final selection of bioindicator species in 2003 was made on-site. The main bioindicators for the 2003 survey included, but were not restricted to: Chinese tallowtree, winged sumac, and wild grapevine. The wild grape species appears to be Vitis labrusca, commonly referred to as "labrusca" grape. Poison-ivy foliage was turning a uniform red (not stippled), making it impossible at times to distinguish ozone-induced stipple. In some locations, Virginia creeper foliage was also turning a uniform red and this species could not be evaluated consistently. Much of the winged sumac had been killed, apparently by herbicides or mowing, and also could not used as a reliable bioindicator. Loblolly pine was not suitable as an indicator species because the large amount of needle browning likely caused by salt damage obscured any ozone-induced chlorotic mottle. Nevertheless, enough suitable bioindicators were present to conduct the 2003 survey.

Foliar Symptoms

Garris Landing - Upland Maritime Forest

2003. In the open areas adjacent to this forested area, 2 of 14 (14.3%) of the labrusca grape plants examined showed possible ozone-induced stipple, but severe insect injury on the leaves confounded this observation (Table 1). The severity of ozone injury was judged to be extremely light (Table 2). Definite ozone injury was not observed on any of the other bioindicators. Elderberry plants, which are generally sensitive to ozone, were no longer growing at locations where they had been evaluated in previous years, probably having been mowed off.

Foliage of scattered devil's club (Aralia spinosa) and Virginia creeper plants was turning a uniform red color; however, this discoloration did not resemble ozone-induced stipple. Occasional Virginia creeper plants were chlorotic and had moderate leafspot infections. Locust leaf miner (insect) injury was common on the foliage of black locust saplings. Labrusca grape leaves were darker green than usual, but had severe skeletonizer and moderate mite injury. Muscadine grape foliage also had severe insect injury and moderate leafspot infections. Sweetgum trees had moderate webworm infestations

In addition, 50 Japanese honeysuckle (Lonicera japonica) plants were examined (as in 1996-1998 and 2002) because of their abundance; these plants were asymptomatic. Also, 50 common ragweed (Ambrosia elatior) and 20 blackberry plants, species known to be sensitive to sulfur dioxide, were examined and found to be free of symptoms resembling those caused by sulfur dioxide. Other broadleaved plant species, whose common and/or scientific names were unknown to the author, were examined. Adaxial stipple, typical of that induced by ozone, was not observed on unknown species growing at the Garris Landing survey site.

Salt marsh cordgrass was examined along the shallow salt marsh near the boat pier. All plants had a chlorotic mottle resembling that caused by ozone in our controlled fumigation experiments. However, this type of symptom on monocots can be caused by various stress factors, including viruses, and should not necessarily be attributed to ozone.

2002. In the open areas adjacent to this forested area, approximately 39% of the labrusca grape plants exhibited ozone-induced stipple in 2002. However, the severity of ozone injury induced on the leaves of the symptomatic individuals in 2002 was extremely light. Approximately 3% of the Virginia creeper plants exhibited very light ozone injury in 2002. Ozone injury was not observed on other bioindicator species.

Bull Island

2003. On Bull Island only 3 of the 116 (2.6%) Chinese tallowtrees exhibited definite ozone injury to the foliage (Table 1). The severity of the ozone injury was light (Table 2). No other bioindicator species exhibited ozone-induced stipple; grape had severe insect injury. Foliage of some plant species, including winged sumac and sweetgum, was turning red. This discoloration somewhat resembled ozone-induced injury on some individual plants, but any ozone stipple was confounded by the discoloration and was not rated. Other herbaceous and shrubby broadleaved plants, whose common and/or scientific names were unknown to the author, were also examined on the island. Adaxial stipple, typical of that induced by ozone, was not observed on these plants.

2002. On Bull Island approximately 25% of the winged sumac shrubs exhibited classic stipple in 2002. The severity of the injury on the sumac leaves was light. Only 5% of the Chinese tallowtrees had ozone injury in 2002, and the severity of the injury was extremely light. Ozone injury was not observed on other bioindicator species in 2002.

Table 1. Comparison of incidence (percentage) of plants exhibiting ozone injury at the Cape Romain National Wildlife Refuge in 1996-1998, and 2002-2003.

Survey Area	Species	Year 1996	1997	1998	2002	2003
Garris Landing						
	Labrusca Grape	*7/29 (24%)	16/33 (48%)	10/23 (44%)	13/33 (39.4%)	2/14 (14.3%)
	Winged Sumac	5/25 (20%)	6/25 (24%)	NOT EVAL	NOT EVAL	NOT EVAL
Bull Island						
	Labrusca Grape	7/27 (26%)	8/21 (38%)	2/10 (20%)	0/8 (0.0%)	0/1 (0.0%)
	Winged Sumac	0/20 (0%)	8/48 (17%)	NOT EVAL	5/25 (25.0%)	NOT EVAL
	Tallowtree	0/273 (0%)	30/107 (28%)	23/105 (22%)	5/108 (4.6%)	3/116 (2.6%)

*First value is number of plants having ozone injury; second value is number of plants examined

Table 2. Severity of ozone-induced injury on leaves of symptomatic plants in 2003.

Species	Plant No.	Leaf Number 1*	2	3	4	5
Grape	1	5**	5	0	0	0
	2	5	0	0	0	0
Chinese Tallowree	1	10**	10	0	0	0
	2	5	5	0	0	0
	3	10	5	0	0	0

*Oldest leaf of the 5 leaves evaluated.

**Severity values = 0, 5, 10, 20, 40, 60, 80, 90, 95, and 100% of leaf tissue injured.

Table 3. Incidence (percentage) of plants exhibiting ozone injury at the Cape Romain National Wildlife Refuge in each year of survey, as compared with monitored ozone levels and drought index.

Year	% Plants Injured Grape	Tallowtree	cumsum60 Ozone*	Palmer Drought Severity Index**
1996	25	0	9,216	(1.90)
1997	44	28	24,437	1.23
1998	36	22	17,424	(1.75)
2002	32	5	22,668	0.27***
2003	15	3	17,641	2.45

*cumsum60 ozone values as of first day of survey

**drought index as of August 1 of each year; parentheses indicate negative values

***note that the drought severity index in the weeks before the 2002 suvey were in the extreme negative range (see Figure 4)

DISCUSSION

Ambient ozone levels. During the summer months prior to the 2003 survey (September 3-5), this part of South Carolina had experienced relatively low ambient ozone levels (Figure 2), as evidenced by cumsum60 ozone data from the EPA AIRS monitoring site within the refuge. By the end of August 2003, the cumsum60 ozone levels had reached only approximately 18,000 ppb.hrs. During the years of survey, ozone levels were greatest in 1997 and least in 1998. Ambient ozone levels in 1996 and 2002-2003 were intermediate. These ambient ozone levels are low, but appear to be slightly above threshold to cause plant injury, with the possible exception of 1998.

The low levels of ambient ozone within the Cape Romain NWR are probably in part related to the clean, daytime sea breezes that the refuge often experiences during the growing season, or perhaps due to the lack of industrial/urban areas upwind. The overall incidence and severity of ozone injury was, accordingly, generally light in the refuge during all survey years, with the possible exception of the level of ozone injury on Chinese tallowtree in 1997 and 1998. Nevertheless, the results of this 2003 survey revealed that ambient levels of ozone was present in high enough levels to cause foliar injury on vegetation within the boundaries of the Cape Romain NWR, a portion of which contains a Class I air quality area.

Bioindicators. The ozone-sensitive species of wild grape, likely Vitis labrusca, exhibited significant levels of ozone injury within the Cape Romain NWR during most survey years, with the exception of 2003. The incidence (frequency) of ozone injury observed on this wild grape species was approximately: 1996 (25%), 1997 (44%), 1998 (36%), 2002 (32%), and 2003 (15%) (Table 1).

Wild labrusca grapes are one of the most useful bioindicators to establish the presence of ozone injury to vegetation in Class I Wilderness Areas at other refuges besides Cape Romain. The investigator has also observed ozone injury on this grape in many surveys conducted in Pennsylvania. It is interesting that "Concord" is the commercial grape variety that is sensitive to ozone in vineyards of the Northeast. Concord grapes were probably selected from wild labrusca grape. The only confounding aspect of using wild grapes as a bioindicator for ozone is their susceptibility to severe insect attack that confounds stipple evaluations.

There are many Chinese tallowtrees on Bull Island, where they thrive in wet, poorly drained areas that often have standing water. There are few tallowtrees at Garris Landing, probably since the soil of this upland forest is at a higher elevation.

The exotic Chinese tallowtree was introduced to the Charleston area as early as the 1830s as an ornamental, and is well established on Bull Island. Chinese tallowtree appears to be the best biomonitor for evaluating ozone injury in the Cape Romain NWR. Since Chinese tallowtree is generally found in or adjacent to standing water, tallowtrees may keep their stomata open during droughts (unless the drought is very severe). The incidence of Chinese tallowtree on Bull Island showing ozone injury was approximately: 1996 (0%). 1997 (28%), 1998 (22%), 2002 (5%) and 2003 (6%) (Table 1). The 1997-1998 observations agree with the results of Anderson et al. (1986), who found ozone injury on many Chinese tallowtrees.

At Garris Landing, winged sumac had ozone injury on 20% of the shrubs examined in 1996, and on 24% of the plants examined in 1997. However, in remaining years the leaves of this sumac species were quite red and the plants were not evaluated. On Bull Island, the respective levels of injury were 0% in 1996, 17% in 1997, and sumacs were not evaluated in remaining years. The winged sumac around the boat landing at Garris Landing had been mowed or treated with herbicide, as they were no longer present in the areas that had been surveyed in earlier years. On Bull Island, 25% of the winged sumac exhibited classic stipple in 2002, but even these plants were turning red. In 2003, the sumac shrubs were also turning red, but 14 individual plants were evaluated. Ozone injury was not observed in winged sumac in 2003. Winged sumac is not a reliable bioindicator species.

Members of the genus Sambucus (elderberry) are generally very sensitive to ozone, and are generally good bioindicators to use for evaluating ozone injury to vegetation. Elderberry plants are quite scarce in the refuge. In fact, no elderberries had been noted within the refuge in 1996. A clump of elderberry plants were observed along a roadside in the Garris Landing area in 1997 (a high ozone year), and all exhibited classic ozone-induced stipple. These specific plants were added as bioindicators. Unfortunately, most of these elderberry plants were mowed in 1998 and perhaps treated with herbicide; ozone injury was present on approximately 12% of the eight remaining plants that year. There were no elderberry plants at this location since. In the future, effort should be made to locate more elderberry plants within the refuge, perhaps with the aid of refuge personnel.

Year-to-year differences in percentage of plants showing stipple may be somewhat related to annual variations in ambient ozone levels, but are confounded by environmental factors such as year-to-year variation in soil moisture. Reduced soil moisture can induce stomatal closure, resulting in less gas (including ozone) uptake and therefore less stipple. The amount of stomatal closure will vary with species. For example, the summer of 2002 was exemplified by a widespread and severe drought (Figure 4). Thus, in spite of the relative high ozone levels (Figure 3), the drought likely reduced gas uptake and resulted in only light ozone injury in the refuge in 2002. The year 2003 had relatively low ozone levels and, in spite of adequate soil moisture, had little ozone injury. Nevertheless, it is apparent that the levels of ambient ozone as monitored in the refuge are sufficient to cause foliar stipple in most years.

Little is known regarding the sensitivity of salt marsh plants to ozone. At Penn State we exposed salt marsh cordgrass (Spartina) to ozone in open-top chambers. Chlorotic mottle and tipburn symptoms, similar to those induced on Spartina by ozone under these controlled conditions, were noted in the refuge. Additional efforts would be needed to accurately determine if indeed field symptoms were caused by ozone, and to quantify the extent and severity of ozone injury on salt marsh vegetation. An important, missing database in the environmental field is the lack of knowledge regarding the incidence and severity of ozone injury to wetlands vegetation, including salt marsh species, and the inherent ozone-sensitivity of species of concern in these critical landscapes.

The results of the 1996-1998 and 2002-2003 surveys revealed that ozone injury was present on vegetation growing within the boundaries of the Cape Romain NWR, a portion of which is a Class I air quality area. The overall incidence and severity of ozone injury among years slightly followed annual variations in levels of ambient ozone, especially with Chinese tallowtree. However, additional datasets are needed before the statistical validity of such relationships can be established. Because of the wide difference in ambient ozone levels and climatic stress among the survey years, and the consistent response of Chinese tallowtree, the FWS might consider surveying the Cape Romain NWR in the future to follow the level of ozone-induced injury on vegetation over time.

These results should prove useful to the FWS when making air quality management decisions, including those related to the review of Prevention of Significant Deterioration (PSD) permits

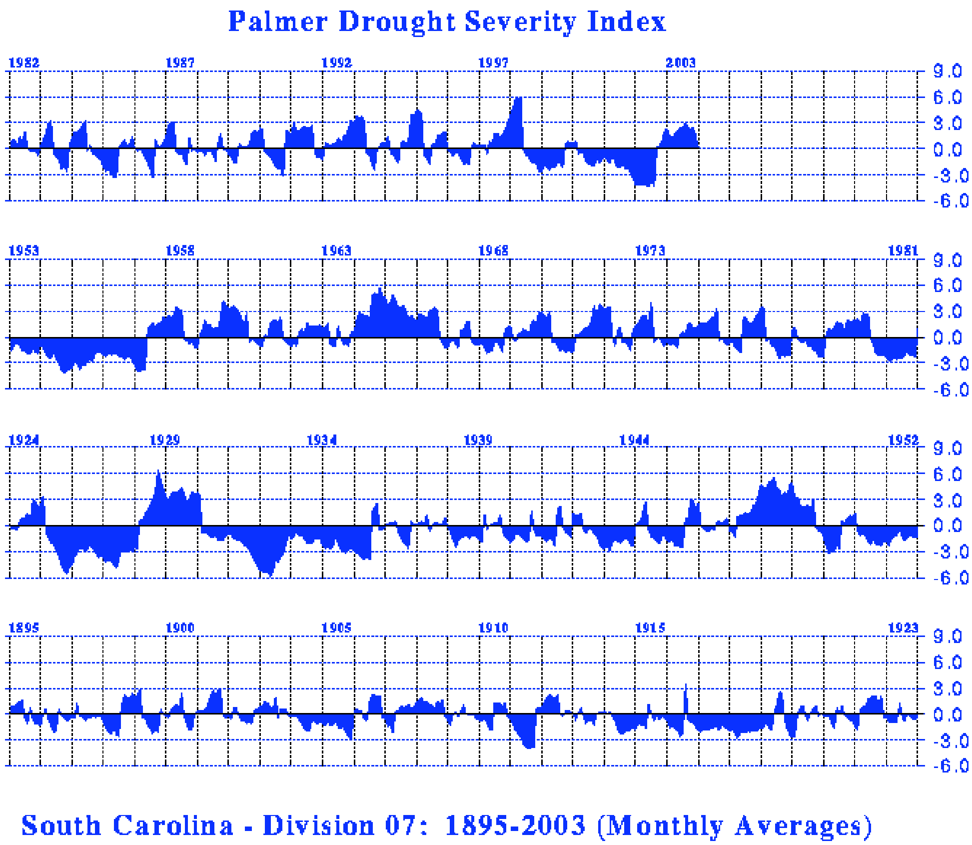

Figure 4. Palmer Drought Severity Index (PDSI) for the coast of South Carolina, including the Cape Romain NWR, during 1895-2003. The horizontal line at "0" is considered normal moisture levels. Areas above the line represent adequate or surplus moisture for normal plant functioning, whereas areas below the line represent potential water stress. A PDSI of -3 is generally considered to be a severe drought, likely reducing ozone uptake**.** The figure illustrates a fairly severe drought in late 2001 and the first part of 2002 and the wet year of 2003.

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APPENDIX

Vegetation List for Cape Romain National Wildlife Refuge Furnished by the US FWS

Scientific Name	Common Name
Acer rubrum L.	(red maple)
Allium L.	(wild onion)
Alternanthera philoxeroides (Mart.) Griseb.	(alligatorweed)
Ambrosia artemisiifolia L.	(annual ragweed)
Ampelopsis arborea (L.) Koehne	(peppervine)
Andropogon L.	(bluestem)
Andropogon virginicus L.	(broomsedge bluestem)
Arundinaria gigantea (Walt.) Muhl.	(giant cane)
Aralia spinosa L.	(devil's walkingstick)
Asclepias L.	(milkweed)
Atriplex L.	(saltbush)
Baccharis halimifolia L.	(eastern baccharis)
Berchemia scandens (Hill) K. Koch	(Alabama supplejack)
Boehmeria cylindrica (L.) Sw.	(smallspike false nettle)
Borrichia frutescens (L.) DC.	(bushy seaside tansy)
Callicarpa americana L.	(American beautyberry)
Cakile P. Mill.	(searocket)
Campsis radicans (L.) Seem. ex Bureau	(trumpet creeper)
Cenchrus echinatus L.	(southern sandbur)
Celtis laevigata Willd.	(sugarberry)
Chenopodium ambrosioides L.	(Mexican tea)
Chloris petraea Sw.	(Eustachys petraea)
Chasmanthium sessiliflorum (Poir.) Yates	(Chasmanthium laxum var. sessiliflorum)
Cinna arundinacea L.	(sweet woodreed)
Cladium jamaicense Crantz	(Cladium mariscus ssp. jamaicense)
Clitoria mariana L.	(Atlantic pigeonwings)
Crataegus L.	(hawthorn)
Croton L.	(croton)
Croton punctatus Jacq.	(gulf croton)
Cyperus grayi Torr.	(Gray's flatsedge)
Cynanchum palustre (Pursh) Heller	(Cynanchum angustifolium)
Cyperus L.	(flatsedge)
Cyperus retrorsus Chapman	(pine barren flatsedge)
Daucus carota L.	(Queen Anne's lace)
Dichromena latifolia Baldw. ex Ell.	(Rhynchospora latifolia)
Digitaria sanguinalis (L.) Scop.	(hairy crabgrass)

Distichlis spicata (L.) Greene	(inland saltgrass)
Diodia teres Walt.	(poorjoe)
Elephantopus tomentosus L.	(devil's grandmother)
Elymus virginicus L.	(Virginia wildrye)
Epilobium angustifolium L.	(fireweed)
Erigeron canadensis L.	(Conyza canadensis var. canadensis)
Erechtites hieracifolia (L.) Raf. ex DC.	(burnweed)
Erigeron philadelphicus L.	(Philadelphia fleabane)
Eupatorium album L.	(white thoroughwort)
Eupatorium capillifolium (Lam.) Small	(dogfennel)
Eupatorium L.	(thoroughwort)
Euphorbia L.	(spurge)
Fimbristylis spadicea auct. non (L.) Vahl	(Fimbristylis thermalis)
Gaura angustifolia Michx.	(southern beeblossom)
Galium L.	(bedstraw)
Galactia volubilis (L.) Britt.	(downy milkpea)
Gelsemium sempervirens St.-Hil.	(evening trumpetflower)
Gnaphalium obtusifolium L.	(Pseudognaphalium obtusifolium ssp. obtusifolium)
Heterotheca subaxillaris (Lam.) Britt. & Rusby	(camphorweed)
Hydrocotyle bonariensis Comm. ex Lam.	(largeleaf pennywort)
Hypericum gentianoides (L.) B.S.P.	(orangegrass)
Ilex glabra (L.) Gray	(inkberry)
Ilex opaca Ait.	(American holly)
Ilex vomitoria Ait.	(yaupon)
Ipomoea L.	(morningglory)
Ipomoea sagittata Poir.	(saltmarsh morningglory)
Iva frutescens L.	(bigleaf sumpweed)
Iva imbricata Walt.	(seacoast marshelder)
Juncus L.	(rush)
Juncus roemerianus Scheele	(needlegrass rush)
Juniperus silicicola (Small) Bailey	(Juniperus virginiana var. silicicola)
Lemna L.	(duckweed)
Limonium carolinianum (Walt.) Britt.	(Carolina sealavender)
Ligustrum japonicum Thunb.	(Japanese privet)
Lippia nodiflora (L.) Michx.	(Phyla nodiflora)
Liquidambar styraciflua L.	(sweetgum)
Lonicera japonica Thunb.	(Japanese honeysuckle)
Matelea carolinensis (Jacq.) Woods.	(maroon Carolina milkvine)
Magnolia grandiflora L.	(southern magnolia)
Melothria pendula L.	(Guadeloupe cucumber)

Mitchella repens L.	(partridgeberry)
Mikania scandens (L.) Willd.	(climbing hempvine)
Myrica cerifera L.	(Morella cerifera)
Oenothera humifusa Nutt.	(seabeach eveningprimrose)
Opuntia compressa J.F. Macbr.	(Opuntia ficus-indica)
Opuntia drummondii Graham	(Opuntia pusilla)
Opuntia P. Mill.	(pricklypear)
Panicum aciculare Desv. ex Poir.	(Dichanthelium aciculare)
Panicum amarum Ell.	(bitter panicgrass)
Paspalum floridanum Michx.	(Florida paspalum)
Passiflora incarnata L.	(purple passionflower)
Panicum L.	(panicum)
Paspalum notatum Fluegge	(bahiagrass)
Panicum oligosanthes J.A. Schultes	(Dichanthelium oligosanthes var. oligosanthes)
Parthenocissus quinquefolia (L.) Planch.	(Virginia creeper)
Paronychia riparia Chapman	(Paronychia baldwinii ssp. riparia)
Panicum virgatum L.	(switchgrass)
Persea borbonia (L.) Spreng.	(redbay)
Phytolacca americana L.	(American pokeweed)
Physalis viscosa ssp. maritima (M.A. Curtis) Waterfall	(Physalis walteri)
Pinus taeda L.	(loblolly pine)
Pluchea camphorata (L.) DC.	(camphor pluchea)
Polygonum hydropiperoides Michx.	(swamp smartweed)
Polygonum L.	(knotweed)
Polypodium polypodioides (L.) Watt	(Pleopeltis polypodioides ssp. polypodioides)
Quercus laurifolia Michx.	(laurel oak)
Quercus virginiana P. Mill.	(live oak)
Rhus copallina L.	(dwarf sumac)
Rhus radicans L.	(Toxicodendron radicans ssp. radicans)
Robinia pseudoacacia L.	(black locust)
Rubus L.	(blackberry)
Rumex crispus L.	(curly dock)
Ruppia maritima L.	(widgeongrass)
Rubus trivialis Michx.	(southern dewberry)
Salsola kali L.	(prickly Russian thistle)
Salicornia L.	(pickleweed)
Sabal minor (Jacq.) Pers.	(dwarf palmetto)
Salix nigra Marsh.	(black willow)
Sabal palmetto (Walt.) Lodd. ex J.A. & J.H. Schultes	(cabbage palmetto)
Sapium sebiferum (L.) Roxb.	(tallowtree)

Sacciolepis striata (L.) Nash	(American cupscale)
Sabatia stellaris Pursh	(rose of Plymouth)
Salicornia virginica L.	(Virginia glasswort)
Scirpus americanus Pers.	(Schoenoplectus americanus)
Scirpus robustus Pursh	(Bolboschoenus robustus)
Scleria triglomerata Michx.	(whip nutrush)
Setaria geniculata Beauv.	(Setaria parviflora)
Setaria magna Griseb.	(giant bristlegrass)
Smilax auriculata Walt.	(earleaf greenbrier)
Smilax bona-nox L.	(saw greenbrier)
Smilax laurifolia L.	(laurel greenbrier)
Smilax rotundifolia L.	(roundleaf greenbrier)
Sonchus asper (L.) Hill	(spiny sowthistle)
Solidago L.	(goldenrod)
Sonchus L.	(sowthistle)
Solidago sempervirens L.	(seaside goldenrod)
Solidago tenuifolia Pursh	(Euthamia tenuifolia var. tenuifolia)
Spartina alterniflora Loisel.	(smooth cordgrass)
Spartina patens (Ait.) Muhl.	(saltmeadow cordgrass)
Teucrium canadense L.	(Candad germander)
Tillandsia usneoides (L.) L.	(Spanish moss)
Toxicodendron radicans (L.) Kuntze	(eastern poison ivy)
Trilisa odoratissima (J.F. Gmel.) Cass.	(Carphephorus odoratissimus)
Triplasis purpurea (Walt.) Chapman	(purple sandgrass)
Typha L.	(cattail)
Ulmus americana L.	(American elm)
Uniola paniculata L.	(seaoats)
Vaccinium arboreum Marsh.	(farkleberry)
Vitis rotundifolia Michx.	(muscadine)
Vitis L.	(grape)
Wisteria frutescens (L.) Poir.	(American wisteria)
Yucca aloifolia L.	(aloe yucca)
Yucca L.	(yucca)
Yucca filamentosa L.	(Adam's needle)
Zanthoxylum clava-herculis L.	(Hercules' club)
Zea mays L.	(corn)
Kosteletskya virginica	()